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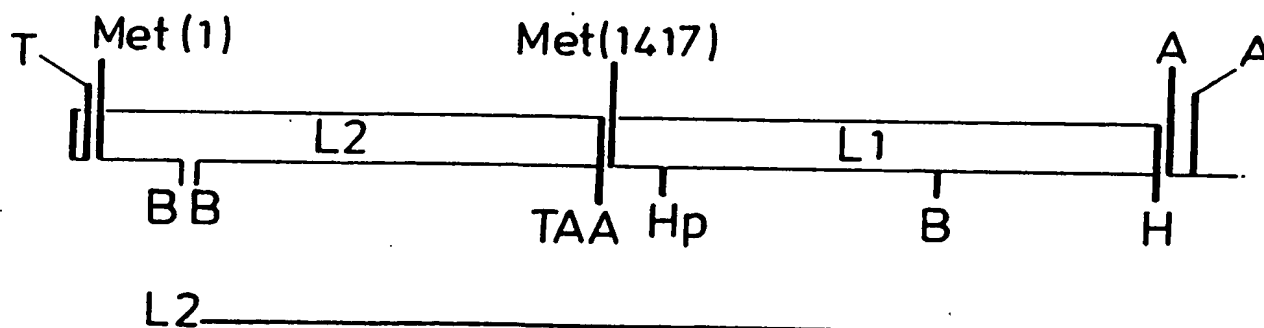


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(21) International Application Number: PCT/GB92/01092 (22) International Filing Date: 17 June 1992 (17.06.92) (30) Priority data: 9113809.9 26 June 1991 (26.06.91) GB (71) Applicant (for all designated States except US): CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED [GB/GB]; 2 Carlton House Terrace, London SW17 5AR (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : JARRETT, William, Fleming, Hoggan [GB/GB]; 60 Netherblane, Blanefield, Glasgow G63 9JP (GB). CAMPO, Maria, Saveria [IT/GB]; 33 Cleveden Road, Glasgow G12 0PH (GB). SMITH, Kenneth, Thomas [GB/GB]; 45 Deepdene Road, Glasgow G61 1NS (GB).			(74) Agent: McCALLUM, William, Potter; Cruikshank & Fairweather, 19 Royal Exchange Square, Glasgow G1 3AE (GB). (81) Designated States: AU, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE). Published With international search report.

(54) Title: PAPILLOMAVIRUS L2 PROTEIN

BPV-2



(57) Abstract

The L2 protein of papillomavirus (particularly BPV-2 and BPV-4) has been cloned as a fusion protein with beta-galactosidase and GST; both as the whole protein and as fragments. Vaccination of calves is found to have both a prophylactic effect in tumour prevention and a therapeutic effect in tumour regression.

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PAPILLOMAVIRUS L2 PROTEIN.FIELD OF THE INVENTION

The present invention relates to the use of papillomavirus L2 protein in medicine, particularly for the regression of papillomavirus tumours in mammals; and to pharmaceutical formulations comprising the L2 protein.

BACKGROUND OF THE INVENTION

Papillomaviruses induce a variety of lesions both in humans and in animals. Some papillomas, albeit benign, are themselves a clinical problem, such as laryngeal papillomas of children (Steinberg and Abramson, 1985) or penile papillomas of bulls (Jarrett, 1985a), and others are known to be a risk factor in the pathogenesis of cancer, as in the case of flat lesions of the cervix or penile condylomata in humans (zur Hausen, 1978). Therefore both in human and veterinary medicine an antiviral vaccine, particularly a therapeutic one inducing lesion rejection, would be of major importance. Vaccination studies in humans present several problems: first of all experimentation is ethically unacceptable and, secondly, very limited amounts of virus are available as some lesions, in particular those of the cervix, do not produce viral progeny, and no in vitro system is yet available which allows vegetative replication of virus.

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The production of viral proteins in bacteria and the use of synthetic peptides have circumvented this last problem and have allowed the ongoing analysis of the immune response to papillomavirus infection (see for instance Jenison et al, 1988; Jochmus-Kudielka et al, 1989; Tindle et al, 1990, Dillner, 1990 and Strang et al, 1990). Whilst investigation into the feasibility of a human papillomavirus vaccine is still at an early stage, effective prophylactic vaccines, both natural (Jarrett et al, 1990a) and genetically engineered (Pilachinski et al, 1986) have already been produced against bovine papillomaviruses, and regression of Shope papillomas has been achieved by vaccinating rabbits with tumour tissue extracts (Evans et al, 1962). The bovine system is an excellent model for the human one, given the several similarities between the two: multiple virus types with high lesion specificity (Campo et al, 1981; Jarrett et al, 1984), homology of genetic structure (Danos et al, 1984) and progression of some lesions to malignancy (Jarrett et al, 1978). The bovine system also presents several advantages: cofactors in oncogenesis are known (Jarrett et al, 1978; Campo and Jarrett, 1986) and, above all, direct experimentation is possible (Jarrett, 1985a).

It has recently been shown that vaccination with bovine papillomavirus type 2 (BPV-2) successfully prevented infection by the same virus (Jarrett et al, 1990a), but not by other virus types (Jarrett et

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al,1990b). Prevention was accompanied by production of neutralising antibodies in the serum of vaccinated animals, indicating that neutralising epitopes are present in the virus.

SUMMARY OF THE INVENTION

Generally speaking, the present invention resides in the discovery that the papillomavirus L2 protein may be prophylactically or therapeutically effective in the treatment of papillomavirus tumours.

Thus, the present invention provides the use of papillomavirus L2 protein in medicine, particularly for the prophylaxis or therapy of papillomavirus tumours.

The invention also provides a pharmaceutical formulation for the prophylaxis or therapy of papillomavirus tumours, which comprises; papillomavirus L2 protein in admixture with a pharmaceutically acceptable carrier.

The invention further provides papillomavirus L2 protein for use in the production of a medicament for use in medicine, particularly for use in the prophylaxis or therapy of papillomavirus tumours.

The invention still further provides a method of treating a mammal for the prophylaxis or therapy of papillomavirus tumours, which comprises the administration of papillomavirus L2 protein to the mammal.

Generally speaking, the prophylactic or therapeutic

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effect of the L2 protein may be limited to the respective papillomavirus type. Thus, for general therapeutic applications, especially where the particular papillomavirus type is unknown, it may be desirable to employ a mixture of L2 proteins from a variety of papillomavirus types.

Generally, the therapy will be applicable to papillomavirus infections of mammals, including humans and animals. In humans, the invention is particularly applicable for the therapy and regression of laryngeal tumours, skin cancer tumours and genital lesions, whether malignant or not. In animals, the therapy is particularly useful for the regression of tumours on animals, for example the removal of warts from the udders of milk cows, or removal of papillomas of the alimentary canal and for the treatment of horses and donkeys. Prophylactic vaccination may also be employed.

The L2 protein is generally produced by recombinant DNA techniques. In particular, a plasmid containing the gene coding for the L2 protein may be transfected into *E. coli* and cultured. The entire L2 protein as it exists in nature may be employed, or a fragment (such as amino acid 90 to 467 of BPV-2 as disclosed hereafter) or fragments thereof may be used providing that the therapeutic effectiveness is retained. The L2 protein may be the native form, with additions, deletions or substitutions which do not substantially effect its therapeutic effectiveness.

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The L2 protein will usually be administered in the form of a pharmaceutical formulation. The formulation contains a pharmaceutically acceptable carrier. The carrier must be acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Since the protein is broken down in the stomach, oral administration is not preferred. The pharmaceutical formulation is preferably formulated for parenteral administration, including subcutaneous, intramuscular and intravenous injection; or as a suppository or pessary. For parenteral administration the formulation may be presented as a sterile solution or suspension in a suitable liquid vehicle, which may also contain preservatives and materials for rendering the formulation isotonic. The formulations may be presented in unit-dose or multi-dose containers. The carrier will generally be apyrogenic. Each dose will generally contain 100 to 10,000 micro grams of the L2 protein.

In order to enhance the therapeutic effect of the protein, it may be administered together with an adjuvant, such as Freund's incomplete adjuvant, as an oil-in-water emulsion or using other adjuvant systems known in the art such as L101 and DDA as used in Pilacinski et al. (1986).

DESCRIPTION OF PREFERRED EMBODIMENTS

Embodiments of the present invention will now be

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described by way of example only with reference to the following experimental protocol.

Figure 1 shows the open reading frames for L2 protein of BPV-2;

Figure 2, 3 and 4 show the results of vaccination experiments using L2 protein of BPV-4 for control group, L2 vaccinated group, and L2 plus E7 vaccinated group respectively.

EXAMPLE 1 (BPV-2 virus)

Figure 1 referred to in the experimental protocol shows the L1 and L2 open reading frames (ORF's) of BPV2 and the restriction enzyme sites used for cloning.

T=TATA box; A=polyadenylation site; Met=translation initiation codon; TAA=translational termination codon; B=BamHI site; Hp=HpaI site; H=HindIII site. The DNA fragment cloned in pUR is indicated as L2 (BamHI-BamHI). The nucleotide numbering of Potter and Meinke (1985) is used.

MATERIALS AND METHODS

Calves

Twenty one 12-week old male Friesian calves were obtained from a papilloma-free source. They were randomly assigned to three initial groups, which were housed in separate, clean, well ventilated pens in the isolation unit of the Department of Veterinary Pathology, Glasgow. All the

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calves were bled on arrival for haematological analysis and to obtain pre-inoculation serum samples. The experiment was started when the calves were 16 weeks old.

Production of L2 peptide of BPV-2 in Escherichia coli

The open reading frame (ORF) encoding the L2 peptide was isolated by digesting the BPV-2 genome cloned in pAT 153 (Campo and Coggins, 1982) with Bam HI. This produced one fragment of 2030 bp (nt 268-2298) numbered according to the nucleotide (nt) sequence of Potter and Meinke (1985), where nt 1 is the A of the ATG codon of the L2 ORF (Figure 1); this fragment contains the majority of the L2 ORF (from aa 90 to aa 467, L2), the L2 ORF stop codon and the 5' half of the L1 ORF, which would not be expressed because of the termination codon. The fragment was cloned in the pUR vector series (Ruther and Muller-Hill, 1983), giving rise to pL2, and transfected into E. Coli JM 109. Peptide for vaccination was prepared from mid-log phase cultures induced for 4 hours in L-broth supplemented with 100 ug/ml ampicillin and containing 1mM IPTG. Bacterial pellets resuspended in lysozyme buffer (50mM TRIS-HCl pH 8.0, 10mM $MgCl_2$, 50mM glucose, 1 mg/ml lysozyme) were left at 20°C for 10 min, when EDTA was added to 50mM. Following cell lysis by the addition of Triton X100 to 1% (v/v), the fusion peptide was pelleted at 39000 g for 30 min and resuspended by boiling and sonication in 5% SDS, 50mM B-mercaptoethanol, 50mM TRIS-HCl, pH 8.0 Purity

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of 90-95% was achieved by preparative SDS PAGE, the final yields being up to 2 mg of product per gm wet weight of cells. The protein was stored at -20°C before use, but prolonged storage caused degradation.

Experimental design

The vaccination experiments were designed as follows:

In group A, six animals were vaccinated prophylactically with the gel-purified L2 (one calf had to be withdrawn from the experiment because of pneumonia); three of these animals were also vaccinated therapeutically with the gel-purified L2 nine weeks after challenge. In group B, eleven animals received no prophylactic vaccination; after tumour formation three of these animals were therapeutically vaccinated with gel-purified L2, while eight animals received no vaccine at all and were therefore used as controls.

Vaccination

The calves receiving the L2 vaccine were given a 1ml PBS suspension containing 650 ug of the L2 fusion protein plus 1 ml of Freund's incomplete adjuvant (FIA) into the right quadriceps muscle. This was repeated fourteen days later as a boost, but with only 500 ug of protein.

Virus challenge

BPV-2 was purified from a skin fibropapilloma (Campo et al, 1981) and the concentration of viral particles was

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estimated by the electron microscope assay (Jarrett et al, 1990a). Each calf was challenged at multiple sites with 10^{12} virus particles as described by Jarrett and other (1990a) either four weeks after vaccination (two weeks after the boost) or nine weeks before vaccination.

Biopsies

Biopsies were performed as described by Jarrett et al (1990a). Immunocytochemical studies were made by the peroxidase-anti-peroxidase (Hsu et al, 1981) or immunogold (Holgate et al, 1983) techniques using rabbit anti-BPV-2 serum as described by Jarrett et al (1984).

Virus neutralization assay

The presence of neutralizing antibodies in serum samples was determined by the cell transformation inhibition assay described previously (Jarrett et al, 1990a). This assay takes advantage of the ability of BPV-2 to transform primary bovine fibroblasts in vitro (Jarrett, 1985b), which is abrogated by pre-incubation of virus with immune serum.

RESULTS

Characterization of fusion protein

The size of the BPV-2 B-gal-L2 fusion protein was estimated on PAGE to be 180 kDa well in agreement with the predicted size of 156 kDa.

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The L2 fusion protein was characterized immunologically. It was injected into rabbits or calves and the antisera were tested against the fusion protein itself and against virion proteins in both Ouchterlony and Western blots assays. The antisera were reactive with both the engineered protein (data not shown) and its viral L2 (62 kDA) counterpart. In reciprocal experiments, rabbit or calf antisera raised against SDS-disrupted virus were reactive with the fusion protein. Although N-terminus truncated, the fusion protein therefore shares epitopes with virus and presents them effectively to the host immune system.

Therapeutic vaccination with BPV-2 L2 fusion protein

Five animals were vaccinated prophylactically; three of these and three unvaccinated animals were vaccinated therapeutically nine weeks after challenge. As the same results were obtained with the two groups of calves, they will be considered together. All animals developed fibropapillomas four weeks after challenge (Table 1). Six vaccinated animals were still bearing tumours at ten weeks. In the other two vaccinated calves the tumours were entering the rejection phase: the epithelium was virtually normal and the sub-epithelial tissue was mainly composed of hyalinised collagen. There was a drastic reduction in the number of fibroblasts and a massive

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infiltration of lymphocytes and macrophages in the sub-epithelial tissue. All vaccinated animals had reached that stage by week thirteen. By week sixteen the tumours had definitely regressed. There were small plaque-like lesions with hyperkeratosis, but virtually all the normal skin adnexal elements were present. Some lymphocytes and macrophages were still present. The control animals were still bearing virus-producing tumours (Table 1). Neutralizing antibodies appeared in the serum of the vaccinated calves at the same time and with the same titre as the control animals (data not shown). Serum antibodies to L2 were however detected soon after vaccination and before challenge (data not shown).

Vaccination with BPV-2 L2 promotes tumour rejection.

Vaccination with the L2 fusion protein, whether delivered before or after challenge, induced early tumour regression. Tumour regression was accompanied by infiltration of the lesion by macrophages and lymphocytes, a process consistently observed when natural regression takes place (Jarrett, 1985a). Thus it appears that the L2 protein encodes epitopes specific for the cellular effector arm of the immune system. Zhou et al (1991) have recently shown that the L1 protein of HPV-16, when expressed in vaccinia virus, induces cytotoxic T-lymphocytes in infected mice, providing another example of T-cell activation by a structural protein.

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In field and experimental cases, rejection takes place approximately twelve months after infection and it generally follows ulceration of the lesion. This is consistent with the L2 being internal to the virion (Jin et al, 1989) and therefore not readily exposed to the host immune system; ulceration of the tumour with associated bleeding would lead to the exposure of relatively large amounts of antigen to the immune cells.

Anti L2 antibodies were present in the serum of the vaccinated animals, but these had no activity in the neutralization assay. Therefore, unless some neutralizing epitopes are present in the first N-terminus amino acids of L2, which are missing in our fusion protein, it is unlikely that L2 plays a significant role in conferring prophylactic protection.

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TABLE 1. Effect of vaccination with BPV-2 L2 fusion proteins.

	WEEKS POST CHALLENGE				
<u>CONTROLS</u>	4	7	10	13	16
1	FP	FP	FP	nd	nd
2	FP	FP	FP	nd	nd
3	FP	FP	FP	nd	nd
4	FP	FP	FP	nd	nd
5	FP	FP	FP	nd	nd
6	FP	FP	FP	nd	nd
7	FP	nd	FP	FP	FP
8	FP	nd	FP	FP	FP
<u>L2 VACCINATES (bc)</u>					
13*	FP	nd	FP	-	-
14	FP	nd	-	-	-
15*	FP	nd	FP	-	-
16	FP	nd	-	-	-
17*	FP	nd	FP	-	-
<u>L2 VACCINATES (ac)</u>					
18	FP	nd	FP	-	-
19	FP	nd	FP	-	-
20	FP	nd	FP	-	-

FP-fibropapilloma; nd=not done; -=no tumours; bc=before challenge; ac=after challenge; animals marked * were vaccinated both before and after challenge.

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EXAMPLE 2 (BPV-4 virus)Production of BPV-4 L2 protein

L2 open reading frame (ORF) of BPV-4 was cloned following the general procedure of Example 1, except that plasmid pGEX was employed which resulted in a L2 fusion protein with glutathione S-transferase (GST) as coprotein. The L2 ORF was cloned as the whole ORF (encoding amino acids 8 to 525) and as the three fragments encoding amino acids 11-201, 203-329, and 330-525. In the subsequent vaccination experiments a mixture of these four was used. Expression was in E.coli and the proteins were purified by gel chromatography, as before.

The E7 protein was prepared in analogous manner.

Vaccination

Vaccination was carried out as in Example 1 using Freund's Incomplete Adjuvant, except that doses of 1mg total protein (L2 and fragments) was administered both as the dose (day 0) and the booster (day 28).

47 calves, of about 10 weeks of age at the start of the experiment, were housed in an isolation compound. They were divided into 2 groups of 15 and one of 17 (controls).

Group 1 was vaccinated with L2 vaccine alone.

Group 2 was vaccinated with L2 plus E7 vaccine.

Group 3 was the control non-vaccinated group.

All animals were examined and bled before Day 0. They

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were vaccinated on Day 0 and Day 28. They were challenged with BPV-4 virus on Day 43 and examined for tumour formation 4 and 7 weeks later.

RESULTS

The results are shown in Figure 2 (controls), Figure 3 (L2 alone) and Figure 3 (L2 plus E7). The controls showed a good tumour response, 13 of the 17 animals being infected. In the L2 vaccinated group only one animal showed a response (a small plaque). In the group vaccinated with L2 plus E7 only one animal developed tumours.

Thus the L2 protein of BPV-4 appears to be exerting a strong prophylactic effect in preventing tumour formation (in contrast to BPV-2 where a therapeutic effect was exhibited).

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CLAIMS

1. A pharmaceutical formulation for the prophylaxis or therapy of papillomavirus tumours, which comprises papillomavirus L2 protein or prophylactically or therapeutically effective fragment thereof in admixture with a pharmaceutically acceptable carrier.
2. A formulation according to claim 1 wherein the L2 protein is a bovine papillomavirus BPV-2 protein or fragment thereof.
3. A formulation according to claim 2 wherein the L2 protein fragment comprises substantially amino acids 90 to 467.
4. A formulation according to claim 1 wherein the L2 protein is a bovine papillomavirus BPV-4 protein or fragment thereof.
5. A formulation according to claim 1 in the form of an injectable formulation, wherein the carrier is a pharmaceutically acceptable injection vehicle.
6. A formulation according to claim 5 which further comprises an adjuvant.

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7. A formulation according to claim 1 wherein the L2 protein is present in the form of a fusion protein with a different co-protein.
8. A formulation according to claim 7 wherein the co-protein in the L2 fusion protein is beta-galactosidase.
9. A formulation according to claim 7 wherein the co-protein is glutathione S-transferase (GST).
10. A formulation according to claim 1 which further comprises papillomavirus E7 protein or effective fragment thereof.
11. A formulation according to claim 1 wherein the L2 protein or fragment thereof is produced by recombinant DNA techniques.
12. A transformed bacterial cell producing recombinant L2 protein or therapeutically effective fragment thereof.
13. Use of papillomavirus L2 protein or effective fragment thereof in medicine for the prophylaxis or therapy of papillomavirus tumours.

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14. Use of papillomavirus L2 protein or therapeutically effective fragment thereof in the production of a medicament for use in the prophylaxis or therapy of papillomavirus tumours.
15. A method of treating a mammal for the therapy of papillomavirus tumours, which comprises the administration of papillomavirus L2 protein or effective fragment thereof to the mammal in a prophylactically or therapeutically effective dosage.

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BPV-2

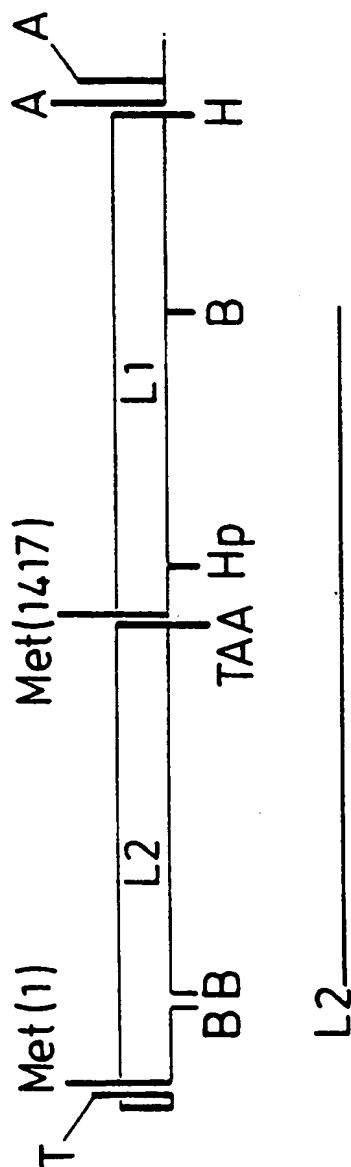


FIG. 1

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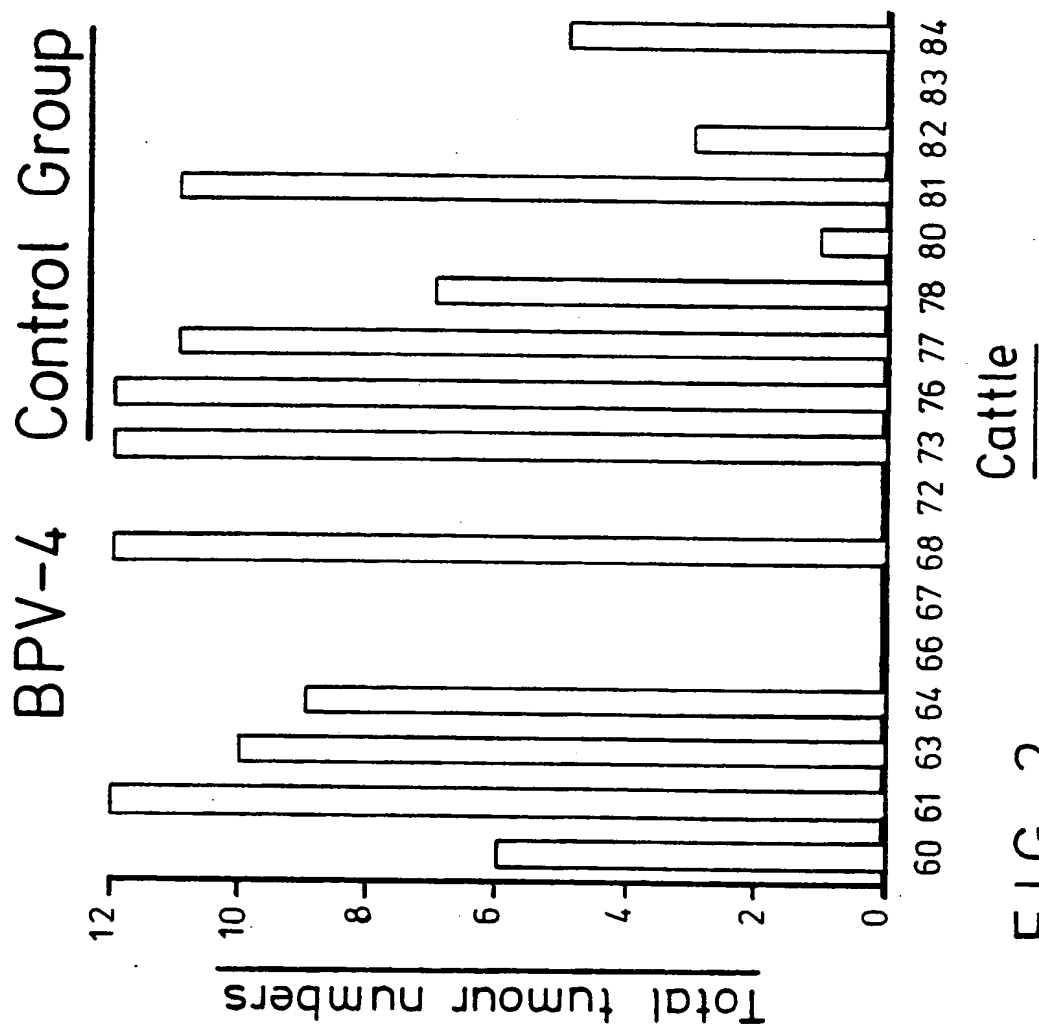


FIG. 2

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BPV-4
L2 Vaccine group

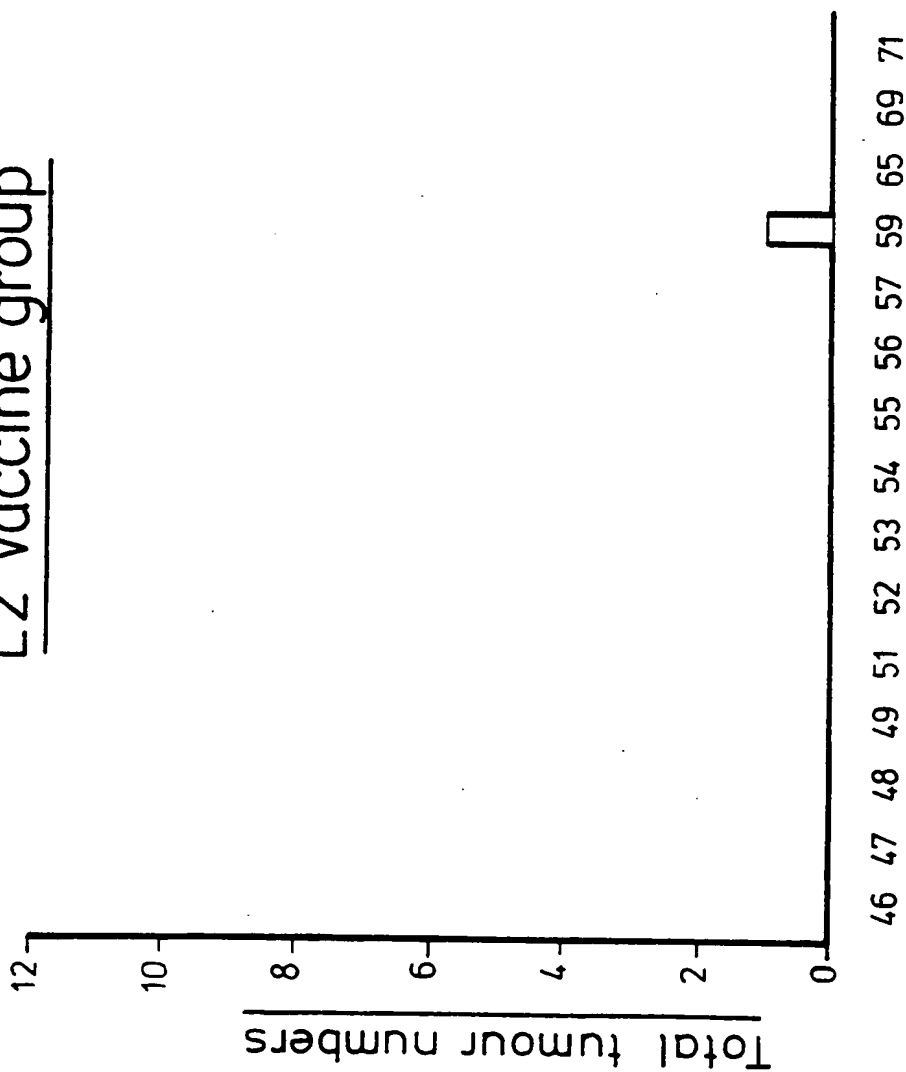
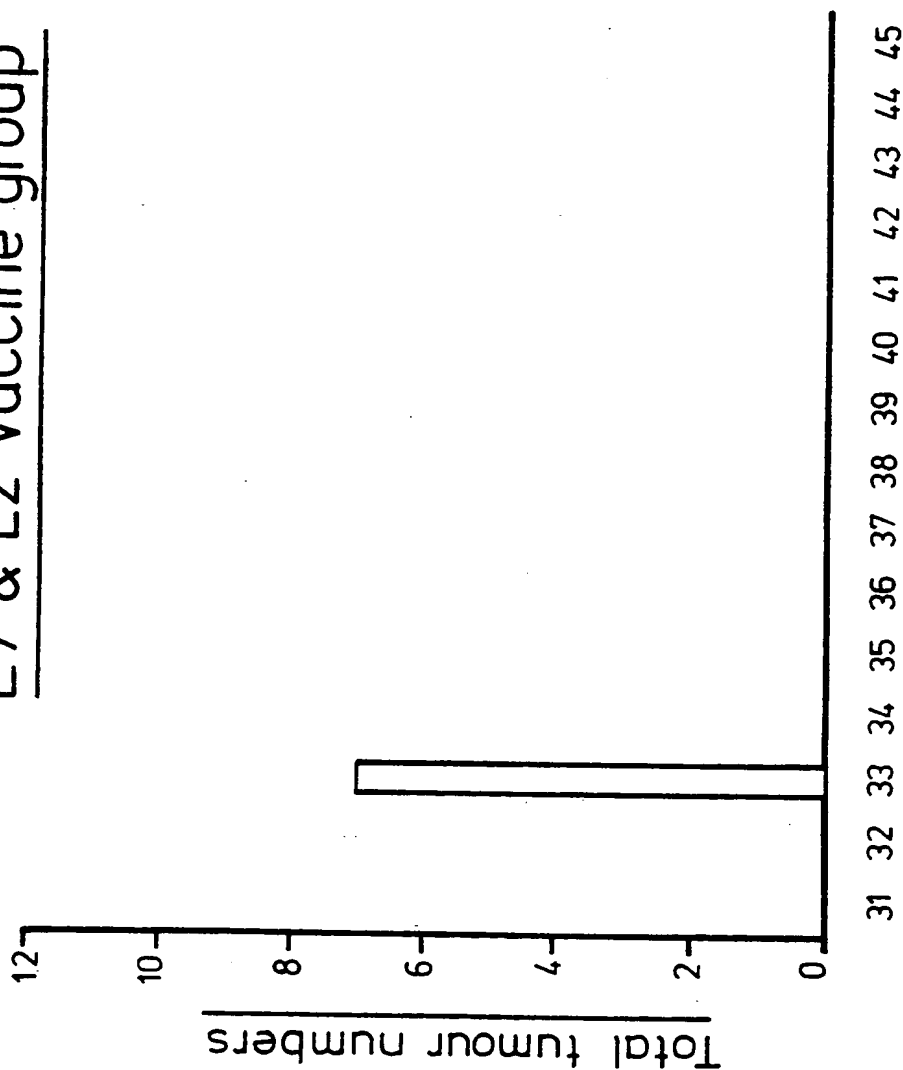


FIG. 3
Cattle

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BPV-4
E7 & L2 Vaccine group



Cattle

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 92/01092

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C12N15/37; C07K13/00; A61K39/12; //C12N15/62		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
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Int.Cl. 5	C07K ; C12N ; A61K	
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III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	EP,A,0 133 123 (MOLECULAR GENETICS, INC.) 13	1,5-8,
Y	February 1985	11-15
	see claims 1-117; figures 1-12	2-4,7,9,
	---	10
X	BIOLOGICAL ABSTRACTS	1,5,6,
	vol. 91, no. 10, PHILADELPHIA, US;	11-15
	page 516;	
	N.D. CHRISTENSEN ET AL.: 'The open reading frame	
	L2 of cottontail papillomavirus contains	
Y	antibody-inducing neutralizing epitopes'	2-4,7,
	abstract no. 107549,	9-10
	& VIROLOGY 181(2): 572-579, 1991	
	abstract	

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¹⁰ Special categories of cited documents: ¹⁰ "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "A" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
01 SEPTEMBER 1992	09. 09. 92	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	HORNIG H.	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category ^o	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claims No.
Y	<p>GENE vol. 67, 1988, ELSEVIER PUBLISHERS, N.Y., U.S.; pages 31 - 40; D.B. SMITH AND K.S. JOHNSON: 'Single step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase' see page 33, left column, line 27 - page 38, left column, line 14; figures 1-4 ---</p>	9
Y	<p>VACCINE vol. 8, no. 3, June 1990, BUTTERWORTH-HEINEMANN LTD., LONDON, pages 199 - 204; G. MENEGUZZI ET AL.: 'Vaccinia recombinants expressing early bovine papilloma virus (BPV1) proteins: retardation of BPV1 tumor development' see page 202, left column, line 19 - page 203, left column, line 44 ---</p>	10
Y	<p>J. GEN. VIROL. vol. 66, no. 1, January 1985, SOC. GEN. MICROBIOL. COLCH., LONDON, pages 187 - 193; H.L. POTTER ET AL.: 'Nucleotide sequence of bovine papillomavirus type 2 late region' cited in the application see page 192, line 24 - line 29; figures 1-4 ---</p>	2,3
Y	<p>J. GEN. VIROL. vol. 68, no. 8, August 1987, SOC. GEN. MICROBIOL. COLCH., LONDON, pages 2117 - 2128; K.R. PATEL ET AL.: 'The nucleotide Sequence and genome organization of bovine papillomavirus type 4' see page 2126, line 10 - line 25; figures 1-4 ---</p>	4
P,X	<p>VIROLOGY vol. 184, no. 1, September 1991, ACADEMIC PRESS, INC. NEW YORK, US; pages 33 - 42; W.F.H. JARRETT ET AL.: 'Studies on vaccination against papillomaviruses: prophylactic and therapeutic vaccination with recombinant structural proteins' see page 34, left column, paragraph 2 - page 41, right column, paragraph 4; figures 1,2; table 1 ---</p>	1-3,5-8, 11-15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB92/01092

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 13,15 are directed to a method of treatment of the human/
animal body the search has been carried out and based on the alleged
effects of the compound/composition
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such
an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

GB 9201092
SA 60676

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 01/09/92

EP-A-0133123	13-02-85	None
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